Influence of Atrazine (2-Chloro-4-Ethylamino-6-Isopropylamino-s-Triazine) on Auxin Metabolism of Plants

Triazine herbicides are classified as specific inhibitors of the Hill reaction[1,2]. Since the introduction of triazines as herbicides in agriculture it has also become evident that side effects on plant growth may occur. A number of papers appeared, reporting triazine-induced growth stimulations[3–13] or growth depressions[7,13,14] in both herbaceous plants and trees, which cannot be explained sufficiently by the aspects of weed control. Apart from the effects on the length of the plants, changes in the size of the leaves[10,15–17] and the stem diameter[9,10,15,18–21] are reported. Furthermore the leaves often look darker green, resulting from an increase in chlorophyll and nitrogen content[3,7,8,10,14,16,19,21,22]. Senescence was shown to be delayed[2,23]. All these data point to the conclusion that triazine herbicides may influence auxin metabolism in plants.

Coleoptile cylinders of oats or cress roots generally react to addition of low levels of atrazine with growth stimulations[24]. Under field conditions triazine herbicides may act as synergists or antagonists to the phenoxyacetic acids depending on the relationship in concentration[25]. Peroxidase was shown to be affected by simazine or atrazine[14,26–27]. As peroxidase is generally thought to participate in the IAA-oxidase system[28,29], it was suggested that atrazine may affect the auxin level of plant tissue by enhancing or retarding IAA destruction. Our studies give support to this hypothesis.

According to the mechanism of an auxin-sparing action, enzyme activity of peroxidase should be inhibited by triazines. This would raise the level of indigenous IAA and result in growth stimulations. A stimulation of enzyme activity, on the other hand, should produce growth reductions by lowering the concentration of the indigenous IAA content in plants.

The IAA-peroxidase activity determination was performed as follows: 4 days old etiolated oat coleoptiles (*Avena sativa*, var. 'Sonnenhafer'), were cut under green light, 3 cm from the apex. The coleoptiles were preincubated for 9 h with atrazine solutions (0.5×10^{-4}; 0.5×10^{-6} to $0.5\times10^{-30}M$), using 1 g of plant material for each concentration. Coleoptiles were then transferred to flasks containing IAA-1-^{14}C in the reaction mixture according to MÜLLER[30] with additional atrazine at the concentrations mentioned above. The flasks were kept in the dark in a water bath (21 °C). Flasks were flushed immediately by decarbonated air and connected with 2 successive traps containing $4N$ KOH. After a reaction time of 14 h, the traps were removed. CO_2 was liberated from the KOH by 50% H_2SO_4 under nitrogen and collected in an organic medium of ethanolamine and methylcellosolve (1:3, v/v). Aliquots were added to the scintillation liquid[31]. Radioactivity was measured with a liquid scintillation counter Tricarb Model 4312. For details of the assay technique see [32]. Radioactivity of $^{14}CO_2$, representing IAA-peroxidase activity, is expressed, after correction for quenching, in percentage of the control. Each point in Figure 1 is the mean from 6 repetitions of the same treatment.

As can be seen from Figure 1, atrazine at concentrations from 10^{-6}–$10^{-8}M$ increased enzyme activity, expressed as the amount of $^{14}CO_2$ released in comparison with the untreated control. Sublethal concentrations of atrazine (0.5×10^{-10} to $0.5\times10^{-21}M$) depressed enzyme activity considerably. The molar concentrations of atrazine, at which inhibition or activation of IAA-peroxidase occur, correspond to the concentrations at which growth stimulations or growth reductions are observed. Hydroxyatrazine was inactive under the experimental conditions.

The influence of atrazine on IAA-peroxidase activity depends on the temperature, as seen from the Table. When incubating the oat coleoptiles with $0.5\times10^{-10}M$ atrazine at 15 °C, IAA-peroxidase activity was strongly inhibited. At 25 °C, however, under the same experimental conditions, IAA-decarboxylation was stimulated. This result can partially be explained by the fact that the uptake of atrazine in plant tissue is known to be increased with rising temperatures[33]. The higher triazine content in the tissue at 25 °C would result in a higher decarboxylation rate. At present, the mechanism of the action of atrazine on IAA-peroxidase remains unexplained. Work will be continued on this aspect.

Influence of temperature on IAA-peroxidase activity under the influence of atrazine

Temperature (°C)	Atrazin	$^{14}CO_2$ released dpm/g per 12 h	%
15	$0.5\times10^{-10}M$	11,113	30
	–	37,107	100
25	$0.5\times10^{-10}M$	45,053	106
	–	42,440	100

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In subsequent work, it was demonstrated that an influence of the triazines on cytokinin metabolism may partly explain the triazine effects on senescence, size of the leaves and stem diameter. JORDAN et al.[34] have demonstrated already a 50% inhibition of tobacco callus growth in dark culture upon addition of $10^{-6}M$ atrazine to the basal medium, which contained 0.05 ppm kinetin. The corresponding hydroxyderivative was inactive. The effects of smaller concentrations of atrazine were not examined by the authors.

As the soybean callus-test is very specific for cytokinin activity, we have continued the experiments with soybean tissue. If atrazine exerts an influence on cytokinin metabolism in plants, growth stimulations should also be observed at adequate herbicide dilutions. Soybean callus tissue was prepared[35] from the cotyledons of Glycine max. var. 'Acme' and subcultured on an nutrient agar medium containing 0.5 ppm kinetin[36] at intervals of 3 weeks (100 ml flasks with 30 ml agar). For the assay, kinetin concentrations were modified and atrazine was added. The slices were grown under diffuse light at 25 °C. They looked yellowish after 3 weeks. 10 repetitions (3 slices per flask) were made for each atrazine concentration. The data (Figure 2) represent the freshweight in percentage of the untreated control. When kinetin was omitted and replaced by atrazine, the tissue did not grow. Therefore, atrazine cannot replace the kinetin and does not act as a cytokinin hormone per se in the plant tissue.

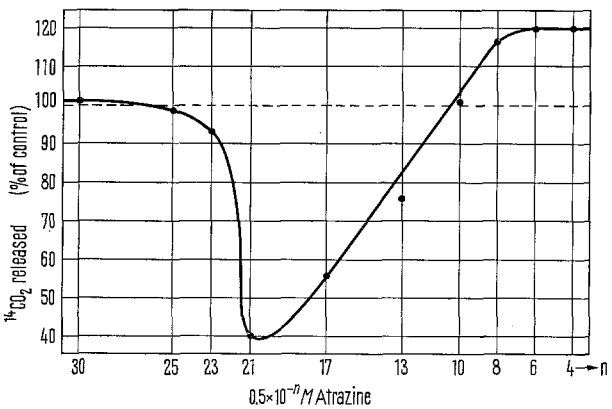

Fig. 1. Influence of atrazine on IAA-peroxidase activity in oat coleoptiles, demonstrated as $^{14}CO_2$ released from IAA-1^{14}C (percentage of untreated control).

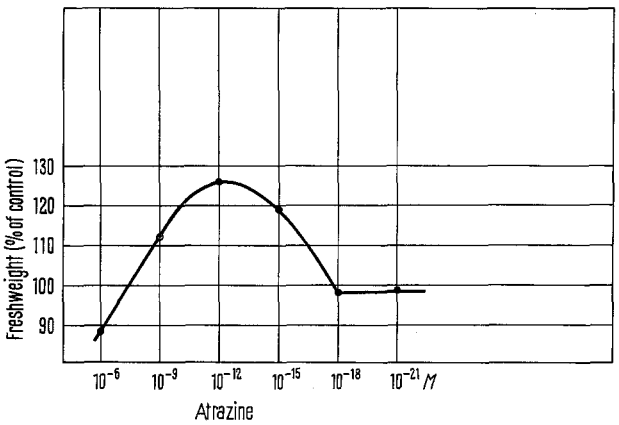

Fig. 2. Growth of soybean tissue on a nutrient agar containing 5 ppm kinetin and various concentrations of atrazine. Growth is shown as percentage of freshweight of control after 21 days.

Upon addition of $10^{-6}M$ atrazine to the basal medium containing 0.05 ppm kinetin, the 50% inhibition of callus growth, which was demonstrated by JORDAN et al.[34] for tobacco, was shown to obtain in soybean tissue, too. Growth reductions were observed with atrazine concentrations down to $10^{-11}M$ at the 0.05 ppm kinetin level. This growth-suppressing effect was diminished and even reversed to a stimulation of cell division at a higher kinetin level (5 ppm) in the nutrient agar, as seen with various concentrations of atrazine (Figure 2). Results are expressed as percentage freshweight when compared with the untreated control cultures. Stimulations of tissue growth were shown to occur at the concentrations ranging from 10^{-8}–$10^{-18}M$ atrazine.

Several authors have already suggested that triazine herbicides may interfere with plant metabolism through a mechanism other than photosynthesis[34,37,38]. The results of our experiments indicate that auxin metabolism is influenced indirectly by atrazine through its action on IAA-peroxidase activity which can partly contribute to an explanation of growth stimulations or growth reductions in plants under the influence of triazine herbicides. Since the amino acid level was reported to be increased under sublethal concentrations of triazines[5,39–41], purin metabolism may also be effected. Activation of cytokinin activity, as demonstrated with the soybean callus tissue, may arise from this relationship.

Assuming that alterations of auxin and cytokinin level in plants are important factors in protein biosynthesis, the influence of atrazine on plant hormones at appropriate concentrations suggest a new basis for a satisfactory explanation of protein increases in treated plants[14,15,42–44].

Zusammenfassung. Die Enzymaktivität der IES-peroxidase in Hafer-Koleoptilen wurde durch Atrazin in Konzentrationen von 10^{-10}–$10^{-21}M$ gehemmt, im Konzentrationsbereich von 10^{-6}–$10^{-8}M$ gesteigert. Atrazin förderte ferner das Wachstum von Sojabohnen-Kallusgewebe in Gegenwart von 5 mg/l Kinetin und 10^{-10} bis $10^{-18}M$ Atrazin im Nähragar. Diese Einwirkung von Atrazin auf den Wuchsstoffmetabolismus der Pflanzen gibt erste Anhaltspunkte, die zahlreich beobachteten Förderungen bzw. Hemmungen des Pflanzenwachstums unter dem Einfluss von Atrazin zu erklären. Die Resultate bilden weiterhin eine neue Grundlage für eine mögliche Interpretation des «Proteineffekts» der Triazine.

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